Expanded View Figures

Figure EV1. Manipulating ESM1 expression is correlated with cell invasion and tumor metastasis (related to Fig 2).

A Relative *ESM1* mRNA expression (left) and ESM1 protein expression (right) in human metastatic PCa cell lines-22Rv1-M and PC3-M transfected with either Scramble-shRNA or ESM1-shRNA. Differences in mRNA levels compared with shScramble cells are shown as fold changes presented as the mean ± SD of three independent experiments. *$P < 0.05$, **$P < 0.01$ when compared to shScramble cells by two-tailed Student's *t*-test. The normalized signal intensity of the target band is shown below the blots as fold changes compared with shScramble group.

B Relative *ESM1* mRNA expression (left) and ESM1 protein expression (right) in human metastatic PCa cells treated with either control or ESM1-locked nucleic acid (LNA). Bars are the mean ± SD of three independent experiments. **$P < 0.01$ when compared to control group by two-tailed Student's *t*-test. The normalized signal intensity of the target band is shown below the blots as fold changes compared with control group.

C Left, representative views of cells in the Transwell invasion assay. Scale bar: 100 μm. Right, quantification of cells that invaded through a Matrigel-coated membrane following treatment with control or ESM1-LNA. Bars are the mean ± SD of three independent experiments. **$P < 0.01$ when compared to control group by two-tailed Student's *t*-test.

D Left, representative views of cells in the Transwell invasion assay. Scale bar: 100 μm. Right, statistical data regarding invasive ability of ESM1-overexpressing cells. Bars are the mean ± SD of three independent experiments. **$P < 0.01$ when compared to vector cells by two-tailed Student's *t*-test.

E Representative images of lungs and livers from mice 8 weeks after orthotopical injection of 22Rv1-P cells transfected with either vector control or ESM1. Scale bar: 0.5 cm. Tumors were removed and weighed. Quantitative summary of tumor luminescence in photons per second of different metastases. *$P < 0.05$, **$P < 0.01$ when compared to vector cells by two-tailed Student's *t*-test and error bars represent the standard deviation of six independent experiments.

Source data are available online for this figure.

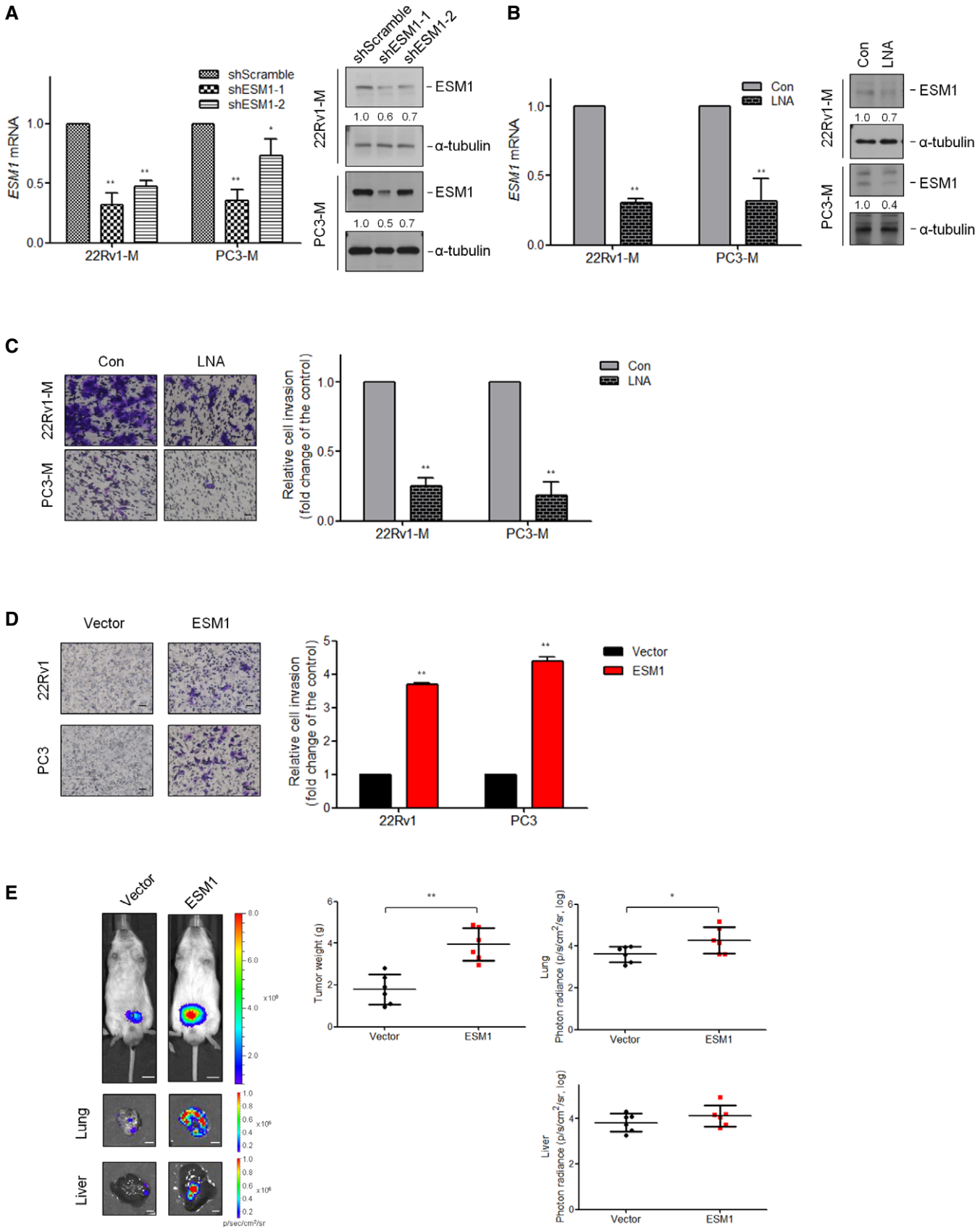
A
ESM1 mRNA
shScramble
shESM1-1
shESM1-2
22Rv1-M
PC3-M
shScramble
shESM1-1
shESM1-2
ESM1
α-tubulin
1.0 0.6 0.7
1.0 0.5 0.7
B
ESM1 mRNA
Con
LNA
1.0 0.7
1.0 0.4
C
Con
LNA
Relative cell invasion (fold change of the control)
D
Vector
ESM1
22Rv1
PC3
E
Tumor weight (g)
Lung
Liver
Photon radiance (p/s/cm²/sr, log)
p/sec/cm²/sr

Figure EV1.

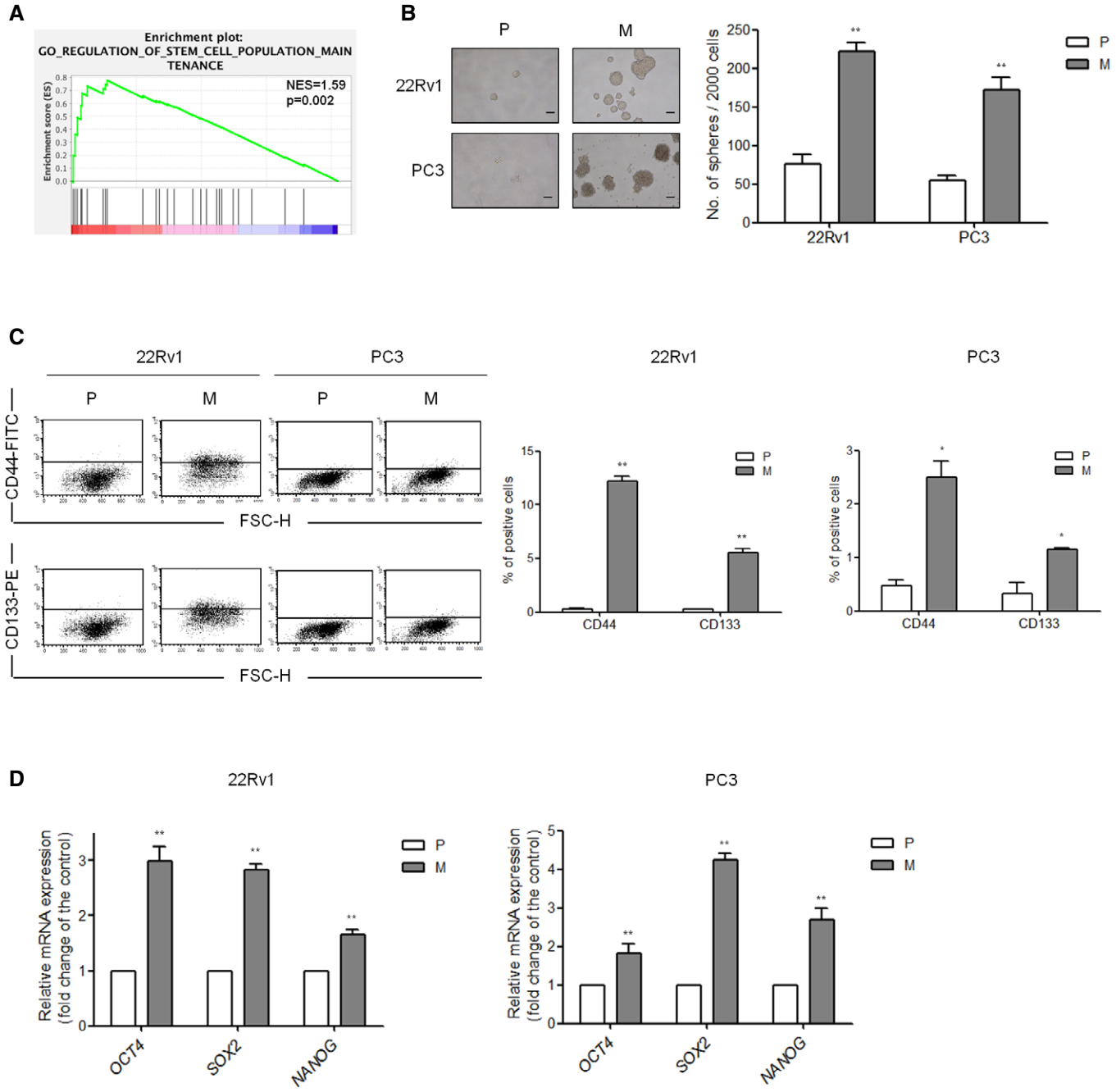

Figure EV2. Characteristics of CSCs are elevated in metastatic PCa cells (related to Fig 3).

A GSEA demonstrating the enrichment of gene sets related to "regulation of stem cell population maintenance" signatures in the ranked gene list of ESM1-high vs. ESM1-low PCa patients from the TCGA database. NES, normalized enrichment score. The *P*-values for the GSEA test statistics are calculated by permutation. The original test statistics for the features are permuted, and new test statistics are calculated for each category, based on the permuted feature test statistics.

B Spheroid formation in 22Rv1-P, 22Rv1-M, PC3-P, and PC3-M cells. P: parental cells, M: metastatic cells. Left, spheroids formed by these four cell lines. Scale bar: 100 μm. Right, total number of spheroid cells was calculated at day 7 in PC3-P and PC3-M cells and at day 14 in 22Rv1-P and 22Rv1-M cells were calculated. Quantitative data comparing the average number of spheres formed are presented as the mean ± SD of three independent experiments. $^{**}P < 0.01$ when compared to parental cells by two-tailed Student's *t*-test.

C Flow cytometry analysis of the ratio of $CD44^{+}$ and $CD133^{+}$ cells in 22Rv1-P/22Rv1-M and PC3-P/PC3-M pairs. Multiples of difference compared with parental cells are presented as the mean ± SD of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ when compared to parental cells by two-tailed Student's *t*-test.

D mRNA expression of PCa stem cell markers, Oct4, Sox2, and Nanog in two sets of human metastatic PCa cell lines. Left, statistical analysis of CSC markers in 22Rv1-P/22Rv1-M pairs. Right, statistical analysis of CSC markers in PC3-P/PC3-M pairs. Differences in mRNA levels compared with parental cells are shown as fold changes presented as the mean ± SD of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ when compared to parental cells by two-tailed Student's *t*-test.

Figure EV3. Manipulating ESM1 expression is correlated with spheroid formation (related to Fig 3).

A Nuclear (N) and cytosolic (C) extracts of adherent (P) and spheroid (S) cells in 22Rv1-P and PC3-P cells were prepared, and the levels of indicated proteins were detected by immunoblotting.

B Representative images of tumorspheres are shown. Spheres were cultured for 14 days in 22Rv1-M cells and 7 days in PC3-M cells before counting. Scale bar: 100 μm. Quantitative data comparing the average number of spheres formed are presented as the mean ± SD of three independent experiments. $^{**}P < 0.01$ when compared to shScramble cells by two-tailed Student's *t*-test.

C Representative images of tumorspheres are shown. 22Rv1-M and PC3-M cells were treated with or without ESM1-LNA, and spheroid formation assay was performed. Histogram shows the mean number of spheres cultured as the mean ± SD. Scale bar: 100 μm. $^{**}P < 0.01$ when compared to control group by two-tailed Student's *t*-test and n = 3 biologically independent samples per group.

D 22Rv1-M cells with stable ESM1 knockdown were transfected with different ESM1-expression pattern constructs, and immunoblotting analysis was performed.

E Cell invasion and spheroid formation assays were performed in the 22Rv1-M cells with different ESM1 expression patterns. Scale bar: 100 μm. Bars are the mean ± SD of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ when compared to shScramble or shESM1-vector cells by two-tailed Student's *t*-test.

Source data are available online for this figure.

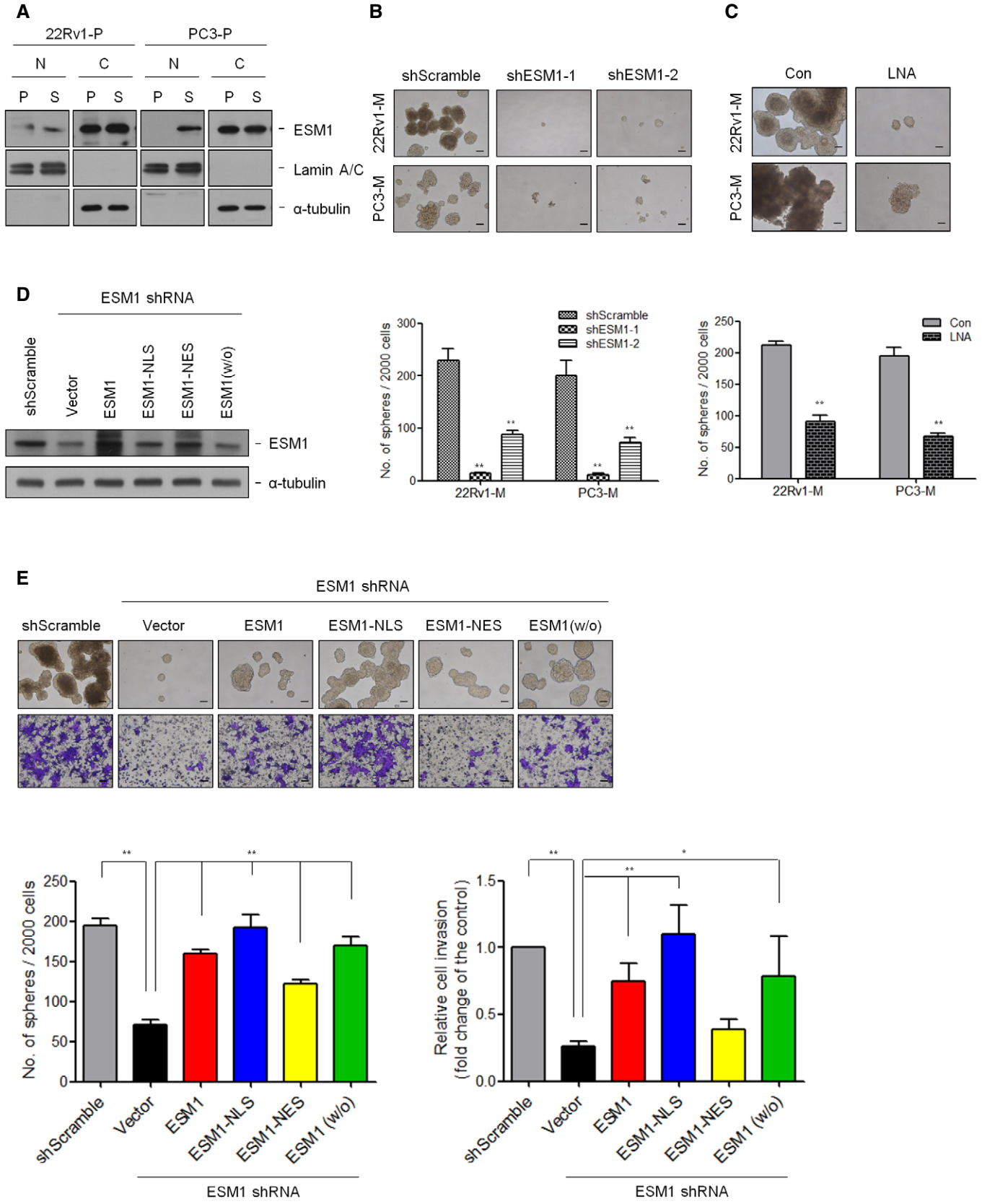

Figure EV3.

Figure EV4. ESM1 mainly induces nuclear translocation of β-catenin (related to Fig 5).

A Nuclear and cytosolic extracts of 22Rv1-P and 22Rv1-M cells were prepared, and the levels of indicated proteins were detected by immunoblotting.
B Nuclear and cytosolic extracts of 22Rv1-M cells transfected with either Scramble-shRNA or ESM1-shRNA were prepared, and the levels of indicated proteins were detected by immunoblotting.
C Suppression of β-catenin or NFκB by shRNA was analyzed.
D, E Immunoblotting analysis of 22Rv1-M cells transfected with either shScramble or shESM1 was performed with the antibodies indicated.
F Nuclear and cytosolic extracts of 22Rv1-M cells transfected with either shScramble or shRELA were prepared, and the levels of indicated proteins were detected by immunoblotting.
G Graph derived from co-expression analysis of published data available in the cBioPortal (EGAS00001002923 and phs000915.v1.p1). *P*-value was obtained by two-tailed Student's *t*-test.

Source data are available online for this figure.

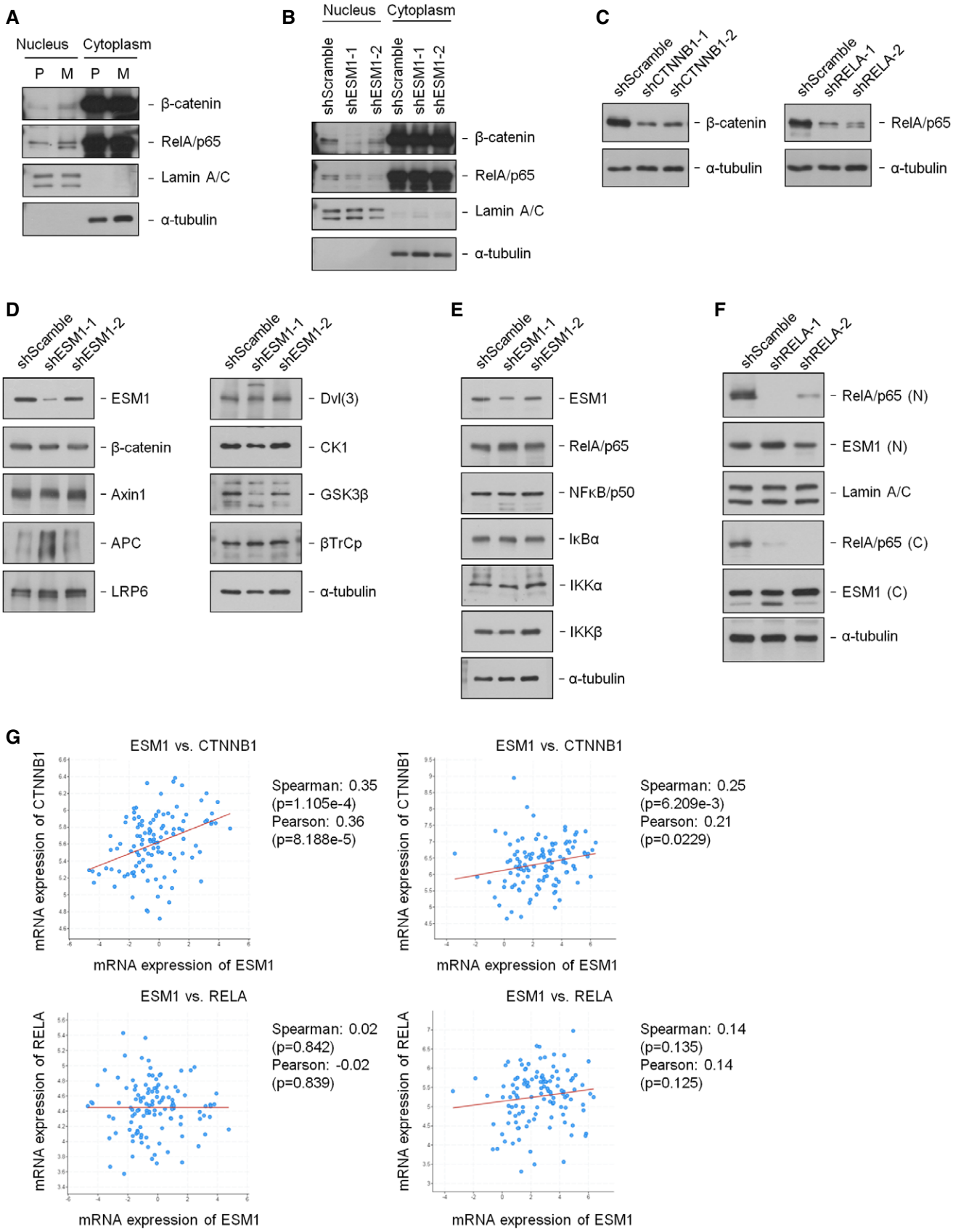

Figure EV4.

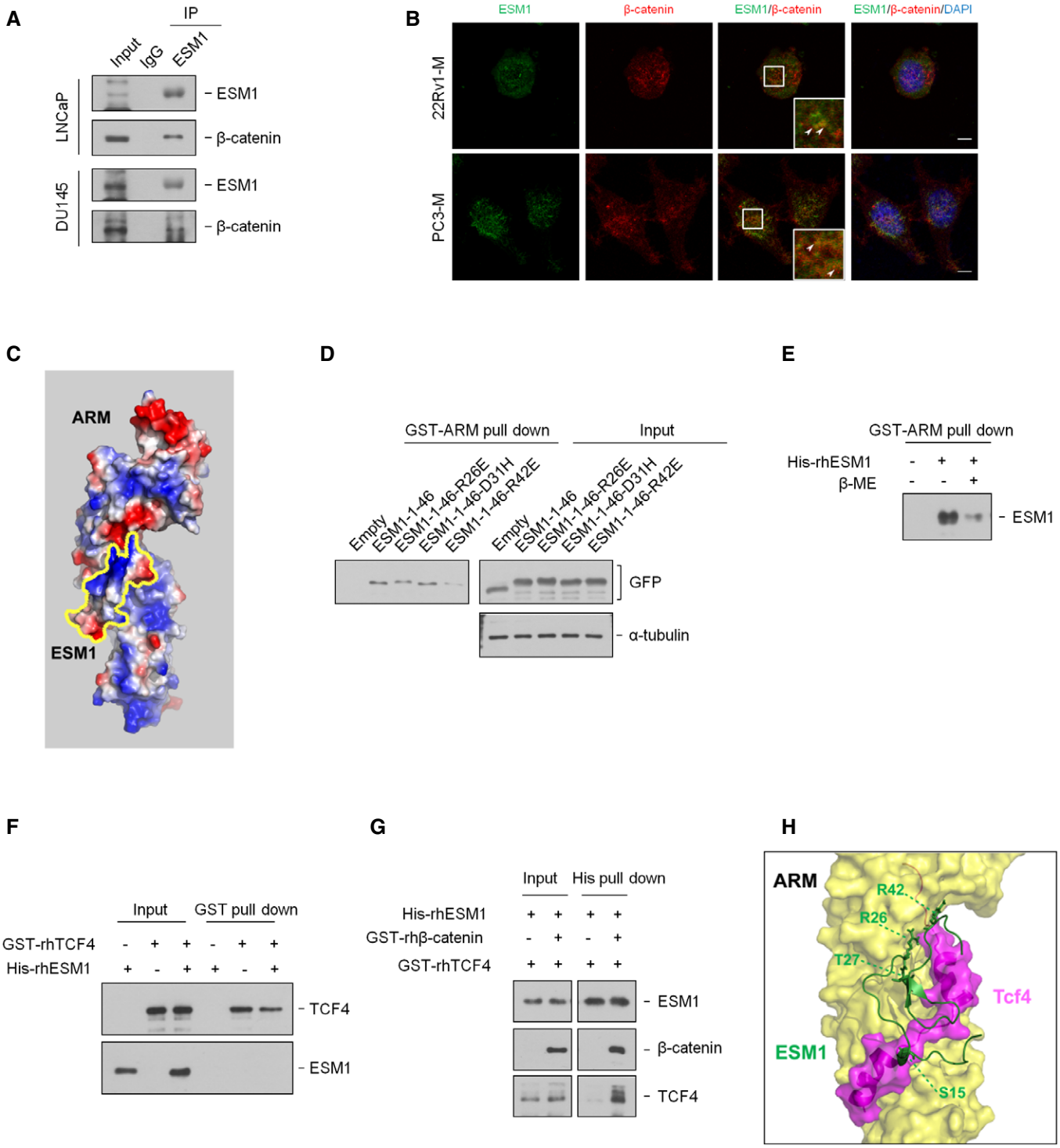
A
IP
Input
IgG
ESM1
LNCaP
ESM1
β-catenin
DU145
ESM1
β-catenin
B
ESM1
β-catenin
ESM1/β-catenin
ESM1/β-catenin/DAPI
22Rv1-M
PC3-M
C
ARM
ESM1
D
GST-ARM pull down
Input
Empty
ESM1-1-46
ESM1-1-46-R26E
ESM1-1-46-D31H
ESM1-1-46-R42E
GFP
α-tubulin
E
GST-ARM pull down
His-rhESM1
β-ME
ESM1
F
Input
GST pull down
GST-rhTCF4
His-rhESM1
TCF4
ESM1
G
Input
His pull down
His-rhESM1
GST-rhβ-catenin
GST-rhTCF4
ESM1
β-catenin
TCF4
H
ARM
R42
R26
T27
Tcf4
ESM1
S15

Figure EV5.

Figure EV5. ESM1 stabilizes β-catenin/TCF4 complex through stereochemically blocking the dissociation of TCF4 from β-catenin (related to Fig 7).

A Cell extracts of LNCaP and DU145 cells were immunoprecipitated with an ESM1 antibody.
B Representative photographs for immunofluorescence staining of endogenous ESM1 and β-catenin in 22Rv1-M and PC3-M cells. Arrowheads indicate the colocalization of ESM1/β-catenin (yellow). Nuclei were counterstained with DAPI. Scale bar: 10 μm.
C Prediction of the surface charge of ESM1 and β-catenin interaction using HADDOCK.
D HEK293T cells were transfected with the indicated ESM1 point mutant constructs. Cell extracts were pulled down with purified GST-ARM.
E Human recombinant His-ESM1 was treated with β-mercaptoethanol and pulled down with purified GST-ARM.
F Human recombinant His-ESM1 and GST-TCF4 proteins were pulled down with glutathione sepharose.
G Human recombinant His-ESM1, GST-TCF4, and GST-β-catenin proteins were pulled down with Ni sepharose.
H Prediction of the ESM1 and β-catenin/TCF4 complex interaction using HADDOCK.

Source data are available online for this figure.